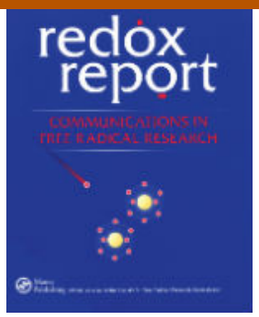

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Research article

Comparison of oxidative stress markers in vaginal deliveries with or without epidural analgesia

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Introduction

Epidural analgesia (EA) is a widely accepted, safe, and reliable method of labour pain relief; it has proved to be beneficial to both mother and child. However, it can be associated with a longer second stage of labour, more frequent oxytocin augmentation, hypotension, and fever, due to changes in the maternal inflammatory reactions, and this may possibly affect the neonatal outcome.[1–3]

For the efficient production of energy, molecular oxygen (O_2) is required as an electron acceptor in all living aerobic organisms. The cell-damaging effects of highly reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), etc. are exerted via a variety of physiological and pathophysiological reactions and have been implicated in many diseases and the process of ageing.[4] Most living organisms have developed well-integrated antioxidant defences to scavenge free radicals. These mechanisms include enzymes, e.g. superoxide dismutases (SODs), catalase (CAT), glutathione peroxidases (GPs), and molecules, e.g. glutathione (GSH), vitamins C and E, and beta-carotene. Oxidative stress may arise when the balance between ROS and antioxidants is disturbed. The ROS can cause intracellular oxidative damage to proteins, nucleic acids, and lipid membranes through the peroxidation of unsaturated fatty acids. The ROS serve as important cell signalling molecules, but in excess they can contribute to the pathophysiology of various diseases associated with the low antioxidant capacity (such as retinopathy and bronchopulmonary dysplasia).[5,6]

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The effects of different analgesia on oxidative balance have already been investigated in numerous articles.[7] The literature seems to agree on the fact that local anaesthetics have potential antioxidant effect.[8,9]

The current study was designed to assess foetal oxidative stress indices in the cord blood of singleton, full-term neonates of mothers who received EA on request as compared with normal vaginal deliveries without the administration of pain control. The level of GSH, the activities of the antioxidant enzymes SOD, CAT, and GP, and the extent of lipid peroxidation (LP) were determined.

Materials and methods

This prospective study, approved by the Ethics Committee at the University of Szeged, involved a total of 86 vaginally delivered singleton infants and their mothers. The parturients were of matched mixed parity in active, spontaneous term labour after an uncomplicated pregnancy. The mothers received full pregnancy care.

The exclusion criteria included the use of any medication, coexisting diseases, instrumental delivery, or caesarean section. The cord blood samples were provided by the Department of Obstetrics and Gynaecology, Medical University of Szeged, Hungary. Eighty-six singleton full-term mature neonates of either sex, born between gestational weeks 37 and 41, were selected, 36 in the EA group, and 50 in the control group.

At enrolment, the level of cervical dilation was 3–6 cm. Only those women were included in the study that gave birth between 3 and 5 hours after enrolment. The participants in the study group received EA on request (epidural group, $n = 36$), whereas the

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mothers in the control group did not desire any pain relief (control group, $n = 50$), except 6 ml of 1% lidocaine before episiotomy.

In the EA group, with the patient in a sitting position, an epidural catheter was inserted, usually to the L3–L4 interspace, using a midline approach with the loss-of-resistance technique. Following a test dose of 3 ml of 2% lidocaine, a bolus dose of 2–3 ml of 0.1–0.2% ropivacaine was administered, followed by a continuous infusion of 20 ml of 0.1% ropivacaine with 0.1 mg of fentanyl at a rate of 4–8 ml per hour during labour. If necessary, a repeated course was given at the patient's request. The basic haemodynamic parameters, body temperature, and cardiotocographic parameters were monitored.

Immediately after delivery, a segment of the umbilical cord was double-clamped, and blood was drawn from the artery into preheparinized plastic syringes in both groups. Coagulation was inhibited with EDTA. For the oxidative stress markers, the duration of storage was kept as short as possible, without adding any preservative with a maximum of a week. The blood samples were centrifuged at 1500 rpm for 10 minutes and the plasma and the buffy coat were removed. The red blood cells (RBCs) were haemolysed after repeated washing with isotonic saline at pH 7.0 by the addition of distilled water in a ratio of 1:9 and were kept on −20°C until processing. With the exception of the SOD activity determinations, the aliquots of the haemolysates were used directly.

Total protein determination

The quantity of proteins was determined with the Folin reagent, bovine serum albumin being used as the standard.[10]

Determination of GSH level

The GSH level of the RBCs was determined with Ellman's reagent.[11] Proteins were precipitated with 5% trichloroacetic acid in order to eliminate protein-linked -SH groups from the measurements.

SOD assay

Before the determination of SOD activity, the haemolysates were treated with ethanol: chloroform (2:1) to remove haemoglobin from the samples, and then centrifuged. The supernatants were used for SOD activity determinations via inhibition of the epinephrine–adrenochrome transformation.[12]

The enzyme activities were calculated from the widely applied (for an enzyme, cell, or microorganism) half maximal inhibitory concentration (IC50) method.

CAT assay

For the CAT assays, erythrocyte haemolysates (100-fold dilution) were used. CAT activity was measured as the H_2O_2 degradation spectrophotometrically at 240 nm. The results were expressed in Bergmeyer units (BUs). One BU is the amount of CAT that decomposes 1000 mg H_2O_2/min.[13]

GP assay

With cumene hydroperoxide and GSH as substrates, GP was determined spectrophotometrically at 412 nm.[14]

LP assay

The LP of the RBCs was determined by the thiobarbituric acid (TBA) method, which reveals the level of total TBA-reactive substances. Calibration was performed with malonyldialdehyde.[15]

Spectrophotometric measurements were made with a Thermo Spectronic Biomate 5 instrument (Thermo Spectronic, Cambridge, UK).

Statistical analysis of the data was performed with Student's *t*-test. A level of $P < 0.05$ was accepted as indicating statistical significance. The Shapiro–Wilks test was applied to confirm the normality of the values. The reported values are means ± SD.

Results

Table 1 depicts the results of measurements relating to the oxidative stress and the activities of antioxidants. The oxidative stress represented by the level of LP was significantly lower in the EA group than in the control group (4.0 ± 1.5 vs. $6.5 \pm 1.8 \times 10^{-2}$ nmol/mg protein, $P < 0.05$). Regarding the antioxidants, the concentration of GSH molecule (5.15 ± 0.48 vs. 7.75 ± 0.63 nmol/mg protein) was also significantly lower in the EA group ($P < 0.05$). Of the antioxidant enzymes, CAT exhibited a significantly lower activity (9.65 ± 0.98 vs. $14.08 \pm 1.2 \times 10^{-4}$ BU/mg protein, $P < 0.01$) in the EA group relative to the control group. The levels of SOD (2.68 ± 0.36 vs. 3.2 ± 0.38 U/mg protein) and GP (3.65 ± 0.43 vs. $4.5 \pm 0.52 \times 10^{-3}$ U/mg protein) were non-significantly lower in the EA group.

Discussion

RBC from neonates born by vaginal delivery with the use of EA were found to display significantly less oxidative stress, but had also significantly lower levels of antioxidant parameters relative to the neonates who underwent normal vaginal delivery without maternal pain relief.

The available data regarding the impact of the delivery mode on the level of oxidative stress of the foetus are inconsistent.[16–19]

The normal neonatal physiological responses to the birth process are complex. In particular, shortly after birth, newborns must adapt to abrupt changes in O_2 concentration and to the increased generation of ROS after their entry into the normoxic environment. The process of birth involves an enhanced degree of oxidative stress for the infant. It is debated whether

Table 1 Effects of EA on the level of LP, on GSH, and on the activities of antioxidant enzymes: SOD, CAT, and GP, relative to the control

	Control ($n = 50$)	EA ($n = 36$)	P
LP	0.065 ± 0.018 nmol/mg protein	0.04 ± 0.015 nmol/mg protein	$P < 0.05$*
GSH	7.75 ± 0.63 nmol/mg protein	5.15 ± 0.48 nmol/mg protein	$P < 0.05$*
CAT	$14.08 \pm 1.2 \times 10^{-4}$ BU/mg protein	$9.65 \pm 0.98 \times 10^{-4}$ BU/mg protein	$P < 0.01$**
SOD	3.2 ± 0.38 U/mg protein	2.68 ± 0.36 U/mg protein	$P > 0.05$
GP	$4.5 \pm 0.52 \times 10^{-3}$ U/mg protein	$3.65 \pm 0.43 \times 10^{-3}$ U/mg protein	$P > 0.05$

*$P < 0.05$.
**$P < 0.01$.

this stress is a necessary event in the foeto–neonatal transition. In 1988, Saugstad conjectured, that there may be a link between extreme oxidative stress and neonatal morbidity.[20] Since then, several studies have suggested a connection between oxidative stress and various neonatal disorders.[21,22] A recent microarray analysis indicated that healthy term foetuses prepare for their impending transition with highly expressed levels of several antioxidant enzymes and associated pathways.[23]

We presume that elevations in stress and antioxidant parameters are normal physiological responses to the process of birth. This stress could also be therefore necessary in the natural process of uncomplicated pregnancies for both the foetus and the mother. In the following, we briefly summarize the most important aspects of this issue. First of all, it has been concluded in several articles that there is a significant relationship between pain and oxidative stress either in animal models or even in preterm infants.[24–26] Accordingly, pain enhances the level of oxidative stress during delivery both in mother and foetus. This increased level of oxidative stress during delivery naturally induces the antioxidative defence mechanism.

Regarding the foetus, there is another interesting maturation phenomenon which has to be mentioned here. If labour occurs at term rather than earlier, it triggers a compensatory up-regulation of the non-enzymatic antioxidant reserve.[27] This up-regulation could be a benefit of labour that protects the newborn from the relative hyperoxia at delivery.

Numerous articles have investigated the effects of different anaesthesia on oxidative balance, and the literature seems to agree on the fact that local anaesthetics have potential antioxidant effect.[28] By considering these data we have to underline that these agents could have attenuated the oxidative stress and enhanced the levels of antioxidants in the EA group; therefore, it could have influenced the differences between our two groups. Unfortunately, as both the mechanism of painkiller drugs and the process of pain-relief result in antioxidative effects, one cannot separate the underlying causes only their combined effects is measurable.

Nevertheless, interestingly enough, the moderation of stress-induced damage through the administration of EA could also be beneficial. Preterm or intrauterine growth retarded infants are especially susceptible to ROS-induced damage, since the state of their antioxidant defence is premature, and their ability to increase the synthesis of antioxidants in response to hyperoxia or other oxidant challenges is inadequate.[29,30]

Our results suggest that EA plays a dual role as concerns oxidative stress, tending to attenuate oxidative stress, but also decreasing the level of antioxidants. Further investigations are definitely needed to evaluate the possible impact of the modulation of oxidative stress during birth.

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